

Certificate of Analysis

PKC iota, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-505, 14-505-K, 14-505M

Parent Lot # 1590317

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant full-length human PKC iota expressed in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 98.3% by SDS-PAGE and Coomassie blue staining. MW = 68.6kDa.

Specific Activity (Parent lot# 1590317): 828U/mg, where one unit of PKC iota, active activity is defined as 1nmol phosphate incorporated into 50μM PKCtide (ERM₁PRKRQGSVRRRV) per minute at 30°C with a final ATP concentration of 100μM.

Formulation: 1.453mg/ml of enzyme in 20mM HEPES/NaOH pH7.4, 250mM NaCl, 50% glycerol, 5mM DTT, 2mM EDTA, 2mM EGTA, 0.05% Triton X-100. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

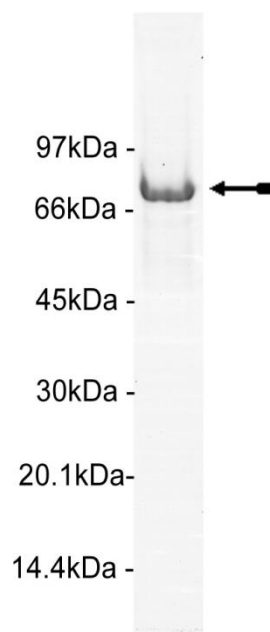
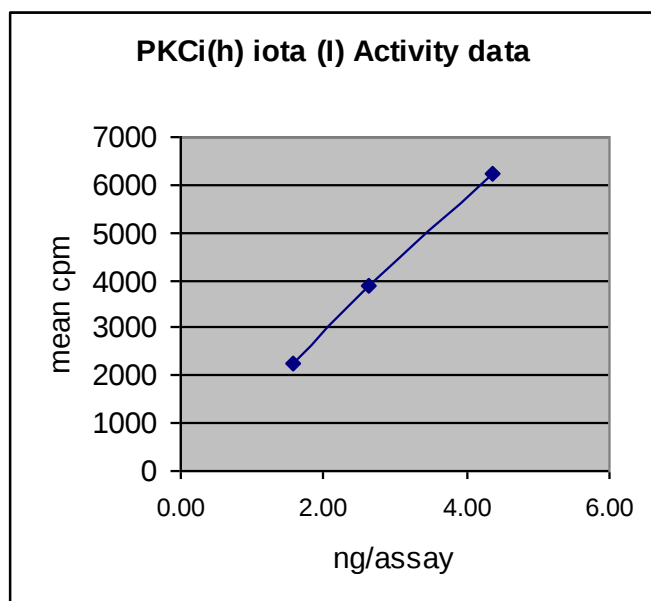
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.6–4.4ng of this lot of enzyme phosphorylated 50μM PKCtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.

MS Tryptic Fingerprint: Confirmed product identity as PKC iota with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of PKC iota, active.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 200mM HEPES/NaOH pH7.4, 0.3% Triton X-100.
2. **PKCtide (ERMRPRKRQGSVRRRV):** Use at a final assay concentration of 50 μ M. Make up a 500 μ M stock. Add 2.5 μ l of stock per assay point.
3. **PKC iota, active:** Dilute with 20mM HEPES/NaOH pH7.4, 0.03% Triton X-100. Use 1.6–4.4ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5 μ l of 10 x reaction buffer per assay to wells.
2. Add 2.5 μ l of **PKCtide (ERMRPRKRQGSVRRRV)**.
3. Add **2.5 μ l (1.6–4.4ng) PKC iota, active**.
4. Add 7.5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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PKC iota Sequence Information

<u>Protein</u>	Human PKC iota
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M10of the recombinant protein is equivalent to M1 of human PKC iota
<u>Accession number</u>	GenBank NM_002740

Recombinant PKC iota amino acid sequence:

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1 MHHHHHHEFM SHTVAGGGSG DSHQVRVKA YYRGDIMITH FEPSISFEGL CNEVRDMCSF
61 DNEQLFTMKW IDEEGDPCTV SSQLELEEF RLYELNKDSE LLIHVFPVCP ERPGMPCPGE
121 DKSIYRRGAR RWRKLYCANG HTFQAKRFNR RAHCAICTDR IWGLGRQGYK CINCKLLVHK
181 KCHKLVITIEC GRHSLPQEPV MPMDQSSMHS DHAQTVIPYN PSSHESLDQV GEEKEAMNTR
241 ESGKASSSLG LQDFDLLRVI GRGSYAKVLL VRLKKTDRYV AMKVVKKELV NDDEDIDWVQ
301 TEKHVFEQAS NHPFLVGLHS CFQTESRLFF VIEYVNGGDL MFHMQRQRKL PEEHARFYSA
361 EISLALNYLH ERGIYRDLK LDNVLLDSEG HIKLTDY GMC KEGLRPGDTT STFCGTPNYI
421 APEILRGEDY GFSVDWWALG VLMFEMMAGR SPFDIVGSSD NPDQNTEDYL FQVILEKQIR
481 IPRSLSVKAA SVLKSFLNKD PKERLGCHPQ TGFADIQGHP FFRNVDWDMM EQKQVPPFK
541 PNISGEFGLD NFDSQFTNEP VQLTPDDDDI VRKIDQSEFE GFYINPLLM SAEECV

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Recombinant PKC iota nucleotide sequence:

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1 atgcatcatc accatcacca tgaattcatg tcccacacgg tcgcaggcgg cggcagcggg
61 gaccattccc accaggtccg ggtgaaagcc tactaccgcg gggatatcat gataacacat
121 tttgaacctt ccattctcctt tgagggcctt tgcaatgagg ttcgagacat gtgttctttt
181 gacaacgaac agctcttcac catgaaatgg atagatgagg aaggagaccc gtgtacagta
241 tcatctcagt tggagttaga agaagccttt agactttatg agctaaacaa ggattctgaa
301 ctcttgattc atgtgttccc ttgtgtacca gaacgtcctg ggatgccttg tccaggagaa
361 gataaatcca tctaccgtag aggtgcacgc cgctggagaa agctttattg tgccaatggc
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1321 gtgctcatgt ttgagatgat ggcaggaagg tctccatttg atattgttgg gagctccgat
1381 aaccttgacc agaacacaga ggattatctc ttccaagtta ttttgaaaaa acaaattcgc
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1501 cctaaggaac gattgggttg tcatcctcaa acaggatttg ctgatattca gggacacccg
1561 ttcttccgaa atgttgattg ggatgatgat gagcaaaaac aggtggtacc tccctttaa
1621 ccaaatattt ctggggaatt tggtttgga aactttgatt ctcagtttac taatgaacct

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1681 gtccagctca ctccagatga cgatgacatt gtgaggaaga ttgatcagtc tgaatttgaa
1741 ggttttgagt atatcaatcc tcttttgatg tctgcagaag aatgtgtctg a

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